

HIGH PRODUCTION VOLUME (HPV)

CHALLENGE PROGRAM

TEST PLAN

For

ALKYL SULFIDE CATEGORY

**Prepared by
The Chemical Manufacturers Association
Petroleum Additives Panel
Health, Environmental, and Regulatory Task Group**

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**LIST OF MEMBER COMPANIES IN THE
HEALTH, ENVIRONMENTAL AND REGULATORY TASK GROUP**

The Health, Environmental, and Regulatory Task Group (HERTG) of the Chemical Manufacturers Association Petroleum Additives Panel includes the following member companies:

Castrol Industrial North America

Chevron Chemical Company, LLC

CK Witco

Ethyl Corporation

ExxonMobil Chemical Company

Ferro Corporation

Infineum

The Lubrizol Corporation

Rhein Chemie Corporation

EXECUTIVE SUMMARY

The Chemical Manufacturers Association (CMA) Petroleum Additives Panel Health, Environmental, and Regulatory Task Group (HERTG), and its member companies, submit for review and public comment their test plan for the “*alkyl sulfide*” category of chemicals under the Environmental Protection Agency’s High Production Volume (HPV) Chemical Challenge Program.

As discussed in the report that follows, these alkyl sulfides, which are used as petroleum lubricant additives, are characterized by having structural similarity and limited reactivity, low biological activity, and very low water volatility. Test data for members of the group show that they are of low concern for aquatic and mammalian toxicity, and, as a result, a reduced testing plan is scientifically justifiable to adequately characterize the category of chemicals.

Alkyl Sulfide Category. Relying on several factors specified in EPA’s guidance document on “Development of Chemical Categories in the HPV Challenge Program,” in which use of chemical categories is encouraged, the HERTG concluded that the following five closely related chemicals constitute a chemical category:

- 2-propanol, 1-(tert-dodecylthio)- (CAS # 67124-09-8, referred to in this report as propanol/dodecylthio derivative)
- 1-decene, sulfurized (CAS # 72162-15-3, referred to in this report as decene derivative)
- 1-propene, 2-methyl-, sulfurized (CAS # 68511-50-2, referred to in this report as methyl propene derivative)
- Pentene, 2,4,4-trimethyl-, sulfurized (CAS # 68515-88-8, referred to in this report as trimethyl pentane derivative)
- Alkenes, C15-18 alpha-, sulfurized, (CAS # 67762-55-4, referred to in this report as C15-C18 alkene derivative).

Structural Similarity. A key factor supporting treatment of these chemicals as a category is their structural similarity. All chemicals in this category consist of hydrocarbon chains (containing fully saturated bonds) with sulfide and polysulfide linkages. None of the chemicals within this category contain reactive (toxic) functional groups. Only one member of the category has a functional group – an alcohol group, which is not expected to be reactive.

Similarity of Physicochemical Properties. The similarity of the *physicochemical properties* of these substances parallels their structural similarity. All are dark colored viscous liquids intended for uses that require stability. The existing database for these substances shows they have limited reactivity, very low water solubility, and low vapor pressure. As a result, the members of the alkyl sulfide category have low potential for hydrolysis, extreme hydrophobicity, very low volatility, and (progressively at increasing molecular weights) limited ability to cross cell membranes. Consequently, they are expected to have low biological activity. What structural variability exists within the category is not expected to result in marked differences in

physicochemical properties, fate and transport characteristics, or in patterns of aquatic or mammalian toxicity. Available data and results of computer modeling support this assessment.

Fate and Transport Characteristics. Based on their physicochemical properties and molecular structures, the HERTG concluded that these chemicals are most likely to adsorb strongly to soil and sediments. To verify this conclusion, the HERTG will develop fugacity data on a number of homologues of the alkyl sulfide category chemicals. These chemicals are also expected to be resistant to hydrolysis and thus, to be stable in water. Compounds in the group are highly hydrophobic such that hydrolysis testing is not technically feasible and the lack of hydrolyzable moieties makes hydrolysis modeling unnecessary. Two of the five alkyl sulfides were subjected to biodegradability testing and found to be poorly biodegradable. The reason for this may be due to the high degree of branching in their alkyl chains. To determine whether there is potential for a higher degree of biodegradability with two of the members of this category that have linear alkyl groups, decene derivative (CAS # 72162-15-3) and C15-C18 alkene derivative (CAS # 67762-55-4), the HERTG will test the decene derivative (CAS # 72162-15-3), with test results to be bridged to two other compounds in the category. Results of this testing will be used to characterize the second material if shown to be significantly different than the existing data. Finally, while it is anticipated that alkyl sulfides will not absorb sufficient sunlight to photodegrade given their tendency to bond to soil, the HERTG plans to develop computer modeled data to adequately characterize the potential atmospheric oxidation potential of this category.

Toxicological Similarity. Review of existing published and unpublished test data for the alkyl sulfide category confirms the *similarity of aquatic and mammalian toxicity* among these five substances. In summary, based on available studies identified by the HERTG, these chemicals demonstrate low concern for aquatic and mammalian toxicity. These findings are expected based on the structure and physicochemical properties of these alkyl sulfide chemicals. In addition, the propanol/dodecylthio derivative (CAS # 67124-09-8) is expected to be the member of the group with the likely upper bound potential for toxicity.

Aquatic Toxicology. Data on acute fish toxicity, acute invertebrate toxicity, and alga toxicity were reviewed. While the HERTG concluded that some additional aquatic toxicity testing is necessary as indicated in the test plan, the findings of available studies generally indicate low acute toxicity to fish and aquatic invertebrates, and low alga toxicity, when environmentally relevant test methods are used.

Mammalian Toxicology - Acute. Data on acute mammalian toxicity (oral, dermal, and inhalation) were reviewed. Oral LD₅₀ levels for all three substances tested were very high, indicating essentially no toxicity, even for the group member (compound in the group) most likely to show the upper bounds of toxicity. Similarly, acute dermal toxicity tests for three of the alkyl sulfide substances, including the compound most likely to show the upper bounds of toxicity, show essentially no toxicity. Inhalation toxicity test data were also reviewed for rats, mice, and guinea pigs. The results were again consistent showing low relative toxicity. The HERTG concluded that:

- the alkyl sulfide category has been generally well tested for acute mammalian effects;
- these tests show low acute toxicity;
- the studies include tests of the compound most likely to represent the upper bounds of acute toxicity; and
- no additional acute mammalian toxicity testing is necessary under the Challenge Program.

Mammalian Toxicology - Mutagenicity. Bacterial reverse mutation assay test data were available for four of the five members of the alkyl sulfide category. In each case the results were negative, both with and without metabolic activation. One of the five members of this category was tested in an *in vitro* chromosomal aberration assay. Again, all results were negative for clastogenicity, both with and without metabolic activation. *In vivo* chromosome aberration studies were available for two of the five alkyl sulfide substances, as well as a structurally similar analogue. All *in vivo* chromosome aberration data reviewed demonstrated that these alkyl sulfides are non-genotoxic, including the chemical in the group with the likely upper bound potential for genotoxicity. Thus, the HERTG concluded that:

- the alkyl sulfide category has been generally well tested for mutagenicity;
- these tests show low concern for mutagenicity;
- the studies include tests of the compound most likely to represent the upper bounds of mutagenicity; and
- no further mutagenicity testing is necessary under the Challenge Program.

Mammalian Toxicology - Subchronic Toxicity. The HERTG reviewed six repeated-dose studies with rats and/or rabbits for three of the five substances, including the substance with predicted upper bound potential for toxicity. No substance-specific toxicity was demonstrated. The changes that did occur in the laboratory animals were determined to be adaptive changes to liver or kidney effects that are not relevant to humans. Because of the consistency of results, the HERTG concluded that:

- the alkyl sulfide category has been generally well tested for repeated dose toxicity;
- these tests show low concern for repeated dose toxicity;
- the studies include a test of the compound most likely to represent the upper bounds of subchronic toxicity; and
- no additional repeated-dose toxicity studies are necessary under the Challenge Program.

Mammalian Toxicology - Reproductive and Developmental Toxicity. Although the alkyl sulfide category is well tested for other mammalian effects, the HERTG was unable to identify any published or unpublished reproductive/developmental studies considered adequate under the Program for compounds in the group. However, since the data on all other endpoints confirm the HERTG's expectation (based on structural similarity and similarity of physicochemical properties) that these chemicals are not biologically active, the HERTG believes no significant reproductive or developmental toxicity will be

demonstrated. Nevertheless, the HERTG plans reproductive and developmental toxicity testing for the propanol/dodecylthio derivative (CAS # 67124-09-8), the member of the group with the likely upper bound potential for toxicity so there will be reproductive and developmental toxicity data considered adequate under the Program for the substances of the group. If this test shows no developmental or reproductive toxicity, the HERTG believes the remaining members of the category would also show no developmental or reproductive toxicity. If this substance yields test results which are positive or equivocal, the HERTG will evaluate the need for additional reproductive testing in this category.

Conclusion. Based on the data reviewed in the report, the HERTG concludes that the physicochemical and toxicological properties of the proposed alkyl sulfide category members are similar and follow a regular pattern as a result of the structural similarity, and therefore meet the EPA definition of a chemical category. As a result, the HERTG believes these five chemicals constitute a category and will test them in accordance with the alkyl sulfide test plan summarized below.

Test Plan. The HERTG's test plan for the alkyl sulfide category includes the following tests and computer modeling:

- Fugacity modeling – propanol/dodecylthio derivative (CAS # 67124-09-8); decene derivative (CAS # 72162-15-3) (mono- and disulfide variants); methyl propene derivative (CAS # 68511-50-2) (lengths y=3 and y=8); trimethyl pentane derivative (CAS # 68515-88-8) (homologues y=1 and y=4); and, C15-C18 alkene derivative (CAS # 67762-55-4 (mono- and disulfide variants)
- Biodegradability study – decene derivative (CAS # 72162-15-3)
- Photodegradation study modeling – propanol/dodecylthio derivative (CAS # 67124-09-8), methylpropene derivative (CAS # 68511-50-2) and C15 – C18 alkene derivative (CAS # 67762-55-4)
- Acute fish toxicity study - propanol/dodecylthio derivative (CAS # 67124-09-8)
- Acute invertebrate toxicity study - propanol/dodecylthio derivative (CAS # 67124-09-8)
- Acute alga toxicity study - propanol/dodecylthio derivative (CAS # 67124-09-8)
- Reproductive/developmental toxicity study - propanol/dodecylthio derivative (CAS # 67124-09-8).

As HERTG developed this test plan, HERTG considered carefully how many animals might be required for tests included in the proposed plan and conditions to which the animals might be exposed. As noted above, a minimal amount of testing requiring use of animals is proposed and, for those tests, HERTG believes the currently available scientific evidence suggests no significant toxicity will be demonstrated. As a result, HERTG believes that the concerns of some non-governmental organizations about animal welfare have been fully considered and that use of animals in this proposed test plan has been minimized.

TABLE OF CONTENTS

LIST OF MEMBER COMPANIES IN THE HEALTH, ENVIRONMENTAL AND REGULATORY TASK GROUP	i
EXECUTIVE SUMMARY	ii
1.0 INTRODUCTION.....	1
2.0 CHEMICAL DESCRIPTION OF ALKYL SULFIDE CATEGORY	3
3.0 EVALUATION OF AVAILABLE PUBLIC AND COMPANY DATA.....	4
3.1 ENVIRONMENTAL FATE DATA.....	4
3.1.1 Fugacity Modeling	4
3.1.2 Hydrolysis	5
3.1.3 Biodegradability.....	6
3.1.4 Photodegradation	7
3.2. ECOTOXICOLOGY DATA	8
3.2.1 Aquatic Ecotoxicity Testing.....	8
3.2.1.1 Test Methodologies	8
3.2.1.2 Test Solution Preparation	9
3.2.1.3 Reporting Toxicity Results	9
3.2.2 Aquatic Toxicity of the Alkyl Sulfide Category.....	10
3.2.2.1 Fish Acute Toxicity	10
3.2.2.2 Invertebrate Acute Toxicity.....	11
3.2.2.3 Alga Toxicity.....	11
3.3 MAMMALIAN TOXICOLOGY DATA	12
3.3.1 Physicochemical Properties Relevant to Mammalian Toxicity.....	12
3.3.2 Acute Mammalian Toxicity of the Alkyl Sulfide Category.....	14
3.3.2.1 Acute Toxicity Test Methodology	14
3.3.2.2 Summary of Available Data	15
3.3.2.2.1 Acute Oral Toxicity	15
3.3.2.2.2 Acute Dermal Toxicity.....	15
3.3.2.2.3 Acute Inhalation Toxicity	16
3.3.2.3 Data Assessment and Test Plan for Acute Mammalian Toxicity	17
3.3.3 Mutagenicity of the Alkyl Sulfide Category.....	17
3.3.3.1 Mutagenicity Test Methodology.....	17
3.3.3.2 Summary of Mutagenicity Data.....	18
3.3.3.2.1 Bacterial Gene Mutation Assay	18
3.3.3.2.2 In vitro Chromosomal Aberration Assay	18
3.3.3.2.3 In vivo Chromosomal Aberration Assays	19
3.3.3.3 Data Assessment and Test Plan for Mutagenicity	19
3.3.4 Repeated Dose Toxicity of the Alkyl Sulfide Category	20

3.3.4.1 Repeated Dose Toxicity Test Methodology	20
3.3.4.1.1 Systemic Organ Studies	20
3.3.4.1.2 Reproductive/Developmental Studies.....	21
3.3.4.2 Summary of Repeated Dose Toxicity Data	21
3.3.4.2.1 Systemic Toxicity Tests	21
3.3.4.2.2 Reproductive/Developmental Toxicity.....	23
3.3.4.3 Data Assessment and Test Plan for Repeated Dose Toxicity.....	23
3.3.4.3.1 Systemic Toxicity	23
3.3.4.3.2 Reproductive/Developmental Toxicity.....	24
TABLE 1. MEMBERS OF THE ALKYL SULFIDE CATEGORY	25
FIGURE 1. CHEMICAL STRUCTURES	25
TABLE 2. FUNCTIONAL GROUP, CHEMICAL CLASSES, AND HYDROLYTIC POTENTIAL OF ALKYL SULFIDE CATEGORY COMPOUNDS	26
TABLE 3. EVALUATION OF ENVIRONMENTAL FATE INFORMATION.....	27
TABLE 4. EVALUATION OF ECOTOXICOLOGY INFORMATION	28
TABLE 5. EVALUATION OF ACUTE TOXICITY INFORMATION	29
TABLE 6. EVALUATION OF MUTAGENICITY INFORMATION.....	30
TABLE 7. EVALUATION OF REPEATED DOSE TOXICITY INFORMATION.....	31

1.0 INTRODUCTION

In March 1999, the Chemical Manufacturers Association (CMA) Petroleum Additives Panel Health, Environmental, and Regulatory Task Group (HERTG), and its participating member companies committed to address data needs for certain chemicals listed under the Environmental Protection Agency (EPA) High Production Volume (HPV) Chemical Challenge Program (Program). This test plan follows up on that commitment.

Specifically, this test plan sets forth how the HERTG intends to address testing information for the five substances listed in Table 1 and identified structurally in Figure 1. These five substances are propanol/dodecylthio derivative (CAS # 67124-09-8); decene derivative (CAS # 72162-15-3); methyl propene derivative (CAS # 68511-50-2); trimethyl pentane derivative (CAS # 68515-88-8); and C15-C18 alkene derivative (CAS # 67762-55-4).

As an integral part of its commitment to the HPV Challenge Program, HERTG has assembled and reviewed available data on these chemicals and determined that they constitute a “chemical category” as provided in the EPA guidance document entitled, “Development of Chemical Categories in the HPV Challenge Program.” The following document provides the basis for that determination, indicates the findings of the data review process, and sets forth a proposed test plan to satisfy parts of the required test battery for endpoints without data that would be considered adequate under the program.

The basis for the HERTG determination that the five substances in this test plan should be treated as a chemical category (i.e., alkyl sulfides) under the HPV Challenge Program is set forth below.

EPA guidance on the HPV Challenge Program indicates that the primary purpose of the program is to encourage “the chemical industry . . . to voluntarily compile a Screening Information Data Set (SIDS) on all chemicals on the US HPV list.” (EPA, “Development of Chemical Categories in the HPV Challenge Program,” p. 1) At the same time, EPA recognizes that the “large number of chemicals to be tested [about 2800 HPV chemicals] makes it important to reduce the number of tests to be conducted, *where this is scientifically justifiable.*” (*Id.*, p. 1) [emphasis added] The next part of the guidance explains where this would be scientifically justifiable:

One approach is to test closely related chemicals as a group, or category, rather than test them as individual chemicals. In the category approach, *not every chemical needs to be tested for every SIDS endpoint.* However, *the test data finally compiled* for the category must prove adequate to support a screening level hazard-assessment of the category and its members. That is, the *final data set* must allow one to estimate the hazard for the untested endpoints, *ideally* by interpolation between and among the category members. In certain cases, such as where toxicity is low and no upward trend is expected, extrapolation to the higher category members may be acceptable. (*Id.*, p. 1) [emphasis added].

EPA guidance goes on to state, “The use of categories is encouraged in the Challenge Program and will have a number of benefits.” (*Id.*, p. 1) Among the benefits identified in the guidance for use of categories are that “a reduction in testing will result in fewer animals used to test a category of chemicals as opposed to doing each test on each individual chemical,” and that “there will be . . . economic savings since less testing may be needed for chemicals considered as a category.” (*Id.*, p. 1) That guidance also states that categories “accomplish the goal of the Challenge Program – to obtain screening level hazard information – through the strategic application of testing to the category.” (*Id.*, p. 2)

A similarly stated intent “to reduce the number of tests to be conducted, *where this is scientifically justifiable*” was articulated by the Agency in its draft guidance document titled, “The Use of Structure Activity Relationships (SAR) in the High Production Volume Chemicals Challenge Program.” [emphasis added].

The EPA “Chemical Categories” guidance sets forth a definition of what constitutes a “chemical category, for the purposes of the Challenge Program”. Specifically, that definition states that a chemical category under the HPV Challenge Program “is a group of chemicals whose physicochemical and toxicological properties *are likely to be similar or follow a regular pattern as a result of structural similarity.*” (*Op. Cit.*, p. 2) [emphasis added].

According to the guidance, what is important is that the “structural similarities [among members of the group] *may create a predictable pattern in any or all of the following parameters: physicochemical properties, environmental fate and environmental effects, and human health effects.*” (*Id.*, p. 2) [emphasis added]. Thus, it is not necessary for the chemicals in a category to be similar in all respects. Nor must there be conclusive proof that the chemicals in the postulated category will behave identically across all relevant parameters. All that is required for an acceptable category under the HPV program is that there be a *likelihood* of similarity of physicochemical and toxicological properties or a *likelihood* that the chemicals will in some pertinent respect follow a regular pattern as a result of their structural similarity.

In identifying the alkyl sulfide category, the HERTG followed the six-step process set out in the EPA guidance on category development. As the following information indicates, the alkyl sulfide category of chemicals put forth by the HERTG clearly satisfies the standards established in that guidance for use of a chemical category:

Step 1: group structurally-similar chemicals into a putative category

Step 2: gather relevant published and unpublished literature for each member of the category

Step 3: evaluate the compiled data for adequacy in accordance with the EPA guidance documentation under the Program

Step 4: construct matrices of SIDS endpoints versus category members arranged so as to indicate the structural progression of the category (in this case, by increasing molecular weight)

Step 5: evaluate the data to determine whether there is a correlation between category members for each SIDS endpoint.

Step 6: make available to EPA, and to the public for review, this test plan including the foregoing category definition and rationale and the following data assessment with the proposed testing scheme for the alkyl sulfide group of chemicals.

2.0 CHEMICAL DESCRIPTION OF ALKYL SULFIDE CATEGORY

The alkyl sulfide category consists of five closely related substances. The chemical names, CAS numbers, and structures for the members of the alkyl sulfide category are presented in Table 1 and Figure 1 (throughout the test plan the chemicals are arranged in the summary tables in order of increasing molecular weight). All five substances are derived from similar starting materials (i.e., alkanes/alkenes and sulfur), and all contain similar organic moieties linked by sulfur with linear, branching, or cyclic structures. Four substances include saturated long-chain hydrocarbons. Two of the substances contain mixtures of linear and cyclic alkyl sulfides. These substances can contain cyclic structures made up of sulfur and carbon, and the alkyl groups can be linear or branched. These structural similarities help explain the similarities in physicochemical properties, environmental fate, ecotoxicity, and mammalian toxicity and establish the justification of this group of materials as a category. Although propanol/dodecylthio derivative (CAS # 67124-09-8) contains a hydroxyl moiety, it is still a long-chain saturated hydrocarbon (a hexane and propyl chain) bridged by a sulfide with side chains consisting of six methyl groups. Consequently, it fits with the expected chemical and biological properties of this category.

The alkyl sulfides are dark colored viscous liquids. These substances range in molecular weight from 260 to 2,300 daltons with an average molecular weight of >500 daltons. As a group, the alkyl sulfides are extremely stable with very low water solubility and volatility. They are essentially unreactive because they are saturated and lack any available π (Pi) electrons that could interact with the nucleophilic center on biological molecules. All but one substance in this category lack functional groups that are potentially reactive or potentially hydrolyzable, such as alkyl halides, amides, thioamides, imines, carbamates, dithiocarbamates, carboxylic acid esters or other carbonyl functional groups, unsaturated carbon-carbon linkages (i.e., double or triple bonds between carbons), lactones, epoxides, urea groups, guanidine groups, nitro groups, nitrilo groups, azoxy groups, aziridines, azides, hydrazine groups, phosphate esters, heterocyclic functional groups, organic sulfate groups, or sulfonic acid esters. The substances in this category are extremely hydrophobic due to the long-chain aliphatic hydrocarbon structure and the absence of hydrophilic functional groups (as supported by the low estimated water solubility range for the members of this category; 0.5 mg/L to less than 0.001 mg/L). The low volatility of these materials is due in part to their high viscosity, low vapor pressure (10^{-3} - 10^{-9} Pa at 20°C) and range of molecular weights.

Due to the similar chemical characteristics of these five substances, they are expected to exhibit similar and predictable environmental fate and transport characteristics and patterns of mammalian and aquatic toxicity. The inherent stability of these structures suggests that they will

resist hydrolysis and photodegradation. The hydrophobic nature and low volatility of these substances suggests that they should partition to soil and sediment in the environment. The low water solubility of these substances suggests that exposure to aquatic organisms would be limited, which would decrease the likelihood of aquatic toxicity. The hydrophobic nature and molecular weights of these substances will decrease the likelihood of systemic toxicity to mammals. The low volatility of these substances limits the amount of these substances that can be inhaled, which will decrease the likelihood of respiratory toxicity. Furthermore, the chemical stability of these substances combined with the lack of an electrophilic moiety will decrease the likelihood of adverse effects that these substances may have upon the genetic material in cells.

3.0 EVALUATION OF AVAILABLE PUBLIC AND COMPANY DATA

3.1 ENVIRONMENTAL FATE DATA

3.1.1 Fugacity Modeling

Fugacity-based multimedia fate modeling compares the relative distribution of chemicals among environmental compartments (i.e., air, soil, sediment, suspended sediment, water, and biota). A widely used model for this approach is the EQC model¹.

There are multiple levels of the EQC model. In the document, “Determining the Adequacy of Existing Data”, EPA states that it accepts Level I fugacity modeling to estimate transport/distribution values. In the same document the Agency states that Level III model data are considered “more realistic and useful for estimating a chemical’s fate in the environment on a regional basis”. The EQC Level I model utilizes input of basic chemical properties, including molecular weight, vapor pressure, and water solubility to calculate percent distribution within a standardized regional environment. EQC Level III uses these parameters to evaluate chemical distribution based on discharge rates into air, water, and soil, in addition to intermediate transport, advection, and degradation rates. EQC Level III will not be proposed for this evaluation because the physical properties of these chemicals do result in emissions or transport to air or water and appropriate emission levels are as yet unknown. Chemical distribution will be evaluated using a Level I model. Data developed using this level can then be used for simple comparative purposes across several chemical classes.

Some of the degradation rates for this class of chemicals will require an additional model to estimate physical/chemical properties from a structure. The model used for this purpose will be EPIWIN, version 3.02², which is also used by EPA and was developed by the Syracuse Research Corporation. EPIWIN includes algorithms for estimating all properties and rates needed for the application of the EQC model.

Nine basic chemical structures will be used for this evaluation and will represent the structures shown in Figure 1. All of the possible structure variations from these nine basic chemical structures will not be modeled, but for a number of chemicals the high and

¹ Equilibrium Criterion Model- Environmental Modeling Centre as developed by D. Mackay.

² Environmental Science Center- Syracuse Research Corporation- EPI for windows.

low molecular weight range will be evaluated in the models, as well as the mono- and disulfide linkages. Specifically, propanol/dodecylthio derivative (CAS # 67124-09-8) will be modeled as shown, and for the decene derivative (CAS # 72162-15-3), both the monosulfide and disulfide homologues will be evaluated. For the methyl propene derivative (CAS # 68511-50-2), dimethyl alkyl sulfide chain (noted as y in Figure 1) of lengths three and eight (i.e., y=3, y=8) will be used and for the trimethyl pentane derivative (CAS # 68515-88-8) methyl alkyl sulfide chain lengths of one and four will be modeled. Finally, for the C15-C18 alkene derivative (CAS # 67762-55-4), mono- and disulfide (straight chain) homologues with ethyl substituents (shown in parentheses in Figure 1) will be modeled.

All of these compounds have very low water solubility and low volatility. They also have very high log K_{ow} values and they bind strongly to organic carbon.

EQC modeling at Level I for the aforementioned chemicals will be conducted as part of the HPV test plan. Based on physical properties, it is expected that these chemicals are most likely to adsorb strongly to soil and sediments.

3.1.2 Hydrolysis

Hydrolysis is a reaction in which a water molecule (or hydroxide ion) substitutes for another atom or group of atoms present in an organic molecule. When an organic molecule undergoes hydrolysis, a nucleophile (water or hydroxide ion) attacks an electrophile and displaces a leaving group (e.g., halogen, phenoxide).³ The lack of a suitable leaving group renders compounds resistant to hydrolysis. Examples of poor leaving groups are alcohols and sulfides. Potentially hydrolyzable groups include alkyl halides, amides, carbamates, carboxylic acid esters and lactones, epoxides, phosphate esters, and sulfonic acid esters (Neely, 1985⁴).

The five compounds in the alkyl sulfide category do not contain functional groups that are subject to hydrolytic reactions and have chemical components with a low potential for hydrolysis (Table 2).

OECD test guideline 111, *Hydrolysis as a Function of pH*⁵, is used to assess the potential for a substance to hydrolyze in water. This test procedure cannot be applied to the HERTG alkyl sulfide category because of the low water solubility and analytical limitations discussed below. Aside from these limitations, determining the hydrolysis potential for these substances is not necessary because they do not contain organic functional groups that are susceptible to this physical degradative mechanism⁶. Therefore, these materials are expected to be stable in water.

³ W. Lyman et al. (1990) *Handbook of Chemical Estimation Methods*. Chapter 8.

⁴ Neely, W.B. (1985) Hydrolysis. In: W.B. Neely and G.E. Blau. Eds. *Environmental Exposure from Chemicals*. Vol.I. CRC Press, Boca Raton, FL, USA. Pp.157-73

⁵ Organization for Economic Cooperation and Development (OECD) (1993) *OECD Guidelines for Testing of Chemicals*. OECD. Paris, France.

⁶ W.J. Lyman, W.F. Reehl, and D.H. Rosenblatt. (1982) *Handbook of Chemical Property Estimation Methods*. McGraw-Hill Book Co. New York, NY, USA.

For substances with water solubilities of less than 2×10^{-2} M, the OECD hydrolysis test procedure requires the preparation of a half saturated aqueous solution of the test substance. For substances in the alkyl sulfide category, this would mean initial test solutions of 0.238 mg/L, based on the most water soluble member of this group of substances [EPIWIN⁷, a computer program, was used to calculate the water solubility of the propanol/dodecylthio derivative (CAS # 67124-09-8)] to less than 0.0002 mg/L for the less water soluble members.

With regard to the test substance, the guideline states that the “analytical method must be sufficiently precise and sensitive to detect a reduction of 10 percent in the initial concentration.” Initial aqueous concentrations of these substances, as required in the test guideline, are likely to be well below the level of analytical detectability. As a result, it would be unlikely that analytical methods would be sufficiently sensitive to detect a 10 percent reduction in test material concentration given the predicted low water solubility of the members in the alkyl sulfide category. As a result, even if these substances were susceptible to hydrolysis, this test could not be performed.

Based on the physicochemical characteristics of the chemicals in this category and the lack of organic functional groups, all the chemicals are expected to be stable in water. Therefore, no testing is necessary.

3.1.3 Biodegradability

Biodegradability is an important factor for determining the fate of chemicals in the environment because it provides a measure for the potential of compounds to be degraded by microorganisms. Chemical biodegradation involves a series of microbially-mediated reactions that may require many kinds of microorganisms acting together to degrade the parent chemical. There are several standard test methods available, each assesses potential biodegradability, based on a measured endpoint of which there are several. There are tests that only measure primary degradation (i.e., loss of parent chemical) or ultimate degradation (i.e., when the chemical is completely utilized resulting in the production of carbon dioxide, water, mineral salts, and microbial biomass). Primary degradation can be determined analytically by measuring dissolved organic carbon (DOC) for water-soluble chemicals by infrared absorbance, or by a chemical-specific method. Ultimate degradation (also called mineralization) is determined by measuring oxygen consumption or carbon dioxide evolution relative to the theoretical levels that can be achieved based on an elemental analysis of the chemical under investigation.

Tests have been developed for measuring biodegradability of chemicals under both aerobic and anaerobic (anoxic) conditions. However, currently only the aerobic tests

⁷ EPIWIN. (1999) Estimation Program Interface for Windows, Version 3.02. Syracuse Research Corporation, Syracuse, NY, USA.

are used in classification and labeling of chemicals in the European Union, which allows for the use of data developed under OECD test guidelines, as well as other sources of standard guidelines.

As depicted in Table 3, two of the members of the alkyl sulfide category have been subject to biodegradability testing.

Propanol/dodecylthio derivative (CAS # 67124-09-8), the most water-soluble member of the category (0.475 mg/L), was subjected to conditions as specified in OECD guideline 301F, Manometric Respirometry Test. In the 28-day test, the measured oxygen demand was 5.9% of theoretical. Based on the test results, this compound exhibited a very slow rate of biodegradability.

Methyl propene derivative (CAS # 68511-50-2), potentially the second most soluble member (≤ 0.000394 mg/L), was subjected to testing conditions as specified in OECD guideline 301B, Modified Sturm Test. In 28 days, 0.3% of the test material was converted to CO₂. Consequently, it was also assessed as exhibiting a very slow rate of biodegradability.

A biodegradability test will be conducted on the decene derivative (CAS # 72162-15-3) to determine if there is potential for a higher degree of biodegradability with two members of this category that have linear alkyl groups; i.e., decene derivative and C15-C18 alkene derivative (CAS # 67762-55-4). If the decene derivative shows significantly different results from the existing data, then the test results will be used to characterize these materials relative to their potential for a more rapid rate of biodegradability.

3.1.4 Photodegradation

Photodegradation is the degradation of a chemical compound as a result of absorption of solar radiation. Therefore, a prerequisite of photodegradation is that one or more bonds of the chemical compound in question has the ability to absorb ultraviolet (UV)/visible light in the 290 to 750 nm range. Light wavelengths longer than 750 nm do not contain sufficient energy to break chemical bonds, and wavelengths below 290 nm are shielded from the earth by the stratospheric ozone layer.

Four of the five substances in this category, including the decene derivative (CAS # 72162-15-3), methyl propene derivative (CAS # 68511-50-2), trimethyl pentane derivative (CAS # 68515-88-8), and C15-C18 alkene derivative (CAS # 67762-55-4), contain a polysulfide bond. These disulfide bonds are capable of absorbing light at a wavelength of 365 nm (i.e., within the wavelength range that may result in breakage of the disulfide bond). However, the strong bonding of these compounds to soil particles resulting from their hydrophobicity may reduce their tendency to absorb sufficient light energy to photodegrade.

The tendency of these alkyl sulfides to photodegrade will be evaluated by using the modeling program AOPWIN. This computer simulation of photo-oxidation is

recommended in the Agency's recently released structure activity review (SAR) guidance for HPV chemicals. Three of the five members of the category will be evaluated to estimate (1) rate constants for the atmospheric, gas phase reaction as mediated by photochemically produced hydroxyl radicals and (2) atmospheric half-lives based on hydroxyl radical concentrations. The members to be modeled are identified in Table 3. Methyl propene derivative (CAS# 68511-50-2) and C15-C18 alkene derivative (CAS# 67762-55-4) represent branched-chain and straight-chain compounds, respectively, that contain polysulfide bonds. Propanol/dodecylthio derivative (CAS #67124-09-8) contains a mono sulfide bond and it is the member with the lowest molecular weight and highest estimated water solubility. The remaining members contain a sulfide bond incapable of light adsorption at the requisite wavelength, so they do not need to be evaluated as they are expected to be non-photodegradable.

3.2. ECOTOXICOLOGY DATA

3.2.1 Aquatic Ecotoxicity Testing

3.2.1.1 Test Methodologies

Three test methodologies are commonly used to conduct aquatic toxicity tests; i.e., flow-through, static, and static renewal tests.

In *flow-through tests*, organisms are continually exposed to fresh chemical concentrations in each treatment level in the incoming water and there is greater assurance than with other test methods that the exposure levels and water quality remains constant throughout the test. Although flow-through testing is the preferred method, it is only applicable for chemicals that have adequate water solubility for testing. The alkyl sulfides cannot be tested by this method because they are all poorly soluble.

In *static tests*, organisms are exposed in still water that is not renewed. The chemical is added to the dilution water to produce the desired test concentrations. Test organisms are then placed in the test chambers and there is no change of water at any time during the test. There is less assurance that the test concentrations test organisms are exposed to will remain constant because test material can be adsorbed onto test chambers, degraded, volatilized, or otherwise changed during the test. Nevertheless, due to limitations of other test systems for non-volatile materials, the static test has been widely used by the scientific community.

The *static-renewal test* is similar to a static test because it is conducted in still water, but the test solutions and control water are renewed periodically, usually every 24 hours. Daily test solution renewal ensures that the exposure concentrations are more likely to be stable throughout the test. This is the preferred method for conducting aquatic toxicity tests for compounds such as the alkyl sulfides on fish. Daily renewals cannot be done in the algae test, and usually not in *Daphnia* tests, because the process of separation and replenishment

would cause a discontinuity in the alga growth rate and it can stress, coat, or entrap *Daphnia* in any surface film during renewals. OECD considers static and static renewal tests appropriate for testing poorly soluble chemicals like the alkyl sulfides provided that test solution preparation use water accommodated fraction or water soluble fraction methods.

3.2.1.2 Test Solution Preparation

Alkyl sulfides are poorly water-soluble substances and it is not possible to prepare exposure solutions for aquatic toxicity testing by direct addition of measured quantities of test material to water. Two methods are used to prepare solutions of poorly soluble materials for aquatic toxicity testing:

- *Water accommodated fraction (WAF)* – This is a method in which the test solution contains only that fraction of the test material (organic phase) which is retained in the aqueous phase after a period of stirring long enough to reach equilibrium, followed by a sufficient time for phase separation. The WAF (aqueous phase) will contain soluble components of the test material at levels that will be dependent on the test material loading (the amount of material added to the aqueous medium). The resulting WAF is used in the aquatic toxicity test. Ideally, a WAF consists of a water-soluble extract of test material, but it can also include a stable micro-emulsion or contain small amounts of suspended matter.
- *Water soluble fraction (WSF)* – This is a method in which a WAF is either filtered, centrifuged, or allowed to settle for a greater length of time (24 hours) than with the WAF method to remove suspended matter from the aqueous phase before being used in the aquatic toxicity test.

3.2.1.3 Reporting Toxicity Results

In both WAF and WSF tests, test material concentrations are expressed as loading rates (i.e., defined as the weight of test material added per unit volume of test medium)⁸. For fish tests, endpoints can be expressed as median lethal loading rate (LL₅₀) when lethal effects occur to 50% of the test population or in cases where no lethal effects are observed at all loadings tested, LL₀. In both cases, results can be expressed in mg/L and in studies where no effects are observed, the result is expressed as LL₀ = the highest loading tested. For invertebrate and alga tests, endpoints are expressed as median effective loading rate (EL₅₀) or EL₀ in mg/L as discussed above.

Loading rates allow poorly water-soluble complex substances such as the alkyl sulfides to be compared to more readily soluble substances and /or pure chemicals on an equal basis (OECD⁸). To allow comparison, the toxicity value is expressed as the amount of test material added per unit volume of water.

⁸ Organization for Economic Cooperation and Development (OECD) (1999) Draft Guidance document on Aquatic Toxicity Testing of Difficult Substances. OECD, France.

If test material exposure levels are analytically measured in the test, the endpoints can also be expressed as median lethal concentration (LC₅₀) or median effective concentration (EC₅₀) in mg/L. EC/LC₅₀s are often not reported because it is very difficult to accurately measure test material exposure levels that can be below 1.0 mg/L.

NOTE: Test results are expressed as EC₅₀ or LC₅₀ even though the tests used WAF or WSF methodology and based on current reporting procedures would be reported as loading rates. In the interest of maintaining consistency between this document and the test reports, the toxicity results have been presented as they were originally reported and converted to lethal loading rate values as described above, which correctly represent the test procedures.

3.2.2 Aquatic Toxicity of the Alkyl Sulfide Category

In general, the toxicity of a substance is limited by absorption into the organism and movement to the target organ(s). Characteristics such as smaller molecule size and a lesser degree of ionization increase the ability of the substance to cross biological membranes. Furthermore, the soluble quantity of a compound in water represents the fraction responsible for toxicity; aquatic toxicity is therefore limited by the water solubility of a compound. For the members of the alkyl sulfide category, the lack of toxicity is due to their low water solubility. Therefore, the substances in the group have limited bioavailability to aquatic organisms. While all group members have low water solubility, relative water solubility is directly linked to alkyl chain length. For equal chain lengths, branched chains are presumed more water soluble than straight chains. Therefore, based on chain length and branching, the lowest molecular weight member of the category, the propanol/dodecylthio derivative (CAS # 67124-09-8) is predicted to be the most water-soluble member of the category and the chemical most likely to represent the upper bounds of aquatic toxicity.

Table 4 summarizes acceptable aquatic toxicity studies and proposed information for the alkyl sulfide category under the HPV Program.

3.2.2.1 Fish Acute Toxicity

The fish acute toxicity test establishes the lethality of a substance to a fish after a 96-hour exposure period. Tests on a member of the alkyl sulfide category assessed in this evaluation were performed in accordance with OECD guideline #203, *Fish, Acute Toxicity Test*.

Because propanol/dodecylthio derivative represents the lower range of molecular size for this category, this material may demonstrate acute toxicity for this endpoint. Therefore, the acute fish toxicity of propanol/dodecylthio derivative (CAS # 67124-09-8) will be determined.

Methyl propene derivative (CAS # 68511-50-2) was tested using WAF and WSF methodology. The results show that this product will not demonstrate acute

toxicity to fish at loading rates of 1,000 mg/L and 10,000 mg/L, as demonstrated using WAF and WSF methodology, respectively. This most likely is due to its high molecular weight and correspondingly low water solubility. These are also characteristics of the three remaining members of this category. Therefore, the acute toxicity data developed for methyl propene derivative (CAS # 68511-50-2) will be used to assess the toxicity of decene derivative (CAS # 72162-15-3); trimethyl pentane derivative (CAS # 68515-88-8); and C15-C18 alkene derivative (CAS # 67762-55-4) to fish.

3.2.2.2 Invertebrate Acute Toxicity

The acute invertebrate test establishes the lethality of a substance to an invertebrate, typically a daphnid (*Daphnia magna*), after a 48-hour exposure period. The tests included in this evaluation (Table 4) were performed in accordance with OECD guideline #202, *Daphnia sp.*, Acute Immobilization Test and Reproduction Test. Acute invertebrate toxicity was tested using WAF methodology.

Because propanol/dodecylthio derivative (CAS # 67124-09-8) represents the lower range of molecular size for this category, this material may demonstrate toxicity for this endpoint. Therefore, the acute invertebrate toxicity of propanol/dodecylthio derivative (CAS # 67124-09-8) will be tested.

In comparison, the remaining members of this category are expected to demonstrate toxicity equivalent to methyl propene derivative (CAS # 68511-50-2) because of their similar higher molecular weights. Existing data for methyl propene derivative (CAS # 68511-50-2) show that this material will not demonstrate acute toxicity to *Daphnia magna* at a loading rate of 1,000 mg/L using the WAF methodology. This is likely due to its high molecular weight and correspondingly low water solubility. Therefore, the acute toxicity data developed for methyl propene derivative (CAS # 68511-50-2) will be used to assess the toxicity of decene derivative (CAS # 72162-15-3); trimethyl pentane derivative (CAS # 68515-88-8); and C15-C18 alkene derivative (CAS # 67762-55-4) to invertebrates.

3.2.2.3 Alga Toxicity

The alga growth inhibition test establishes the potential of a substance to inhibit alga growth, typically using the freshwater unicellular green algae, *Pseudokirchneriella subcapitata* (formerly called *Selenastrum capricornutum*), after a 96-hour exposure period. The test included in this evaluation (Table 4) was performed in accordance with OECD guideline #201, *Alga, Growth Inhibition Test*. Alga growth inhibition was evaluated using WAF methodology. The results are depicted in Table 4.

No data are available for propanol/dodecylthio derivative (CAS # 67124-09-8). Because propanol/dodecylthio derivative (CAS # 67124-09-8) represents the lower range of molecular size for this category, it is anticipated that this material

is the most likely to demonstrate toxicity. Therefore, it is proposed that the acute alga toxicity of propanol/dodecylthio derivative (CAS # 67124-09-8) be determined.

Methyl propene derivative (CAS # 68511-50-2) was tested using WAF methodology. Existing data for methyl propene derivative (CAS # 68511-50-2) show this chemical demonstrates toxicity to alga at loading rates greater than 100 mg/L using WAF methodology. The remaining members of this category are expected to demonstrate toxicity equivalent to methyl propene derivative (CAS # 68511-50-2) because of their similar higher molecular weights and correspondingly low water solubility. Therefore, the toxicity data developed for methyl propene derivative (CAS # 68511-50-2) will be used to assess the toxicity of decene derivative (CAS # 72162-15-3); trimethyl pentane derivative (CAS # 68515-88-8); and C15-C18 alkene derivative (CAS # 67762-55-4) to alga.

3.3 MAMMALIAN TOXICOLOGY DATA

3.3.1 Physicochemical Properties Relevant to Mammalian Toxicity

Typically, for a xenobiotic⁹ to be biologically active it must be able to:

- cross biological membranes in order to reach target tissue/cells in an organism (e.g., either by passive diffusion or active transport via carrier proteins). According to basic principles of pharmacology and toxicology, lipophilicity generally enhances the ability of chemicals to cross biological membranes (i.e., lipophilic compounds are more readily absorbed across biological membranes than hydrophilic or charged chemical moieties). However, for large lipophilic compounds, molecular size becomes a critical limiting determinant (i.e., small lipophilic compounds more readily traverse biological membranes than do large lipophilic materials);
- be transported within the systemic circulation to target cells, provided the target cells are not located at the initial site of entry or contact. Contrary to absorption, which is favored by lipophilicity, basic tenets of pharmacology and toxicology maintain that systemic distribution or transport within the body is enhanced or facilitated by hydrophilicity (i.e., a compound that is hydrophilic may be more readily transported via plasma). At the same time, hydrophilicity enhances urinary excretion and decreases the biological half-life of a xenobiotic within an organism
- be biologically active or be activated by enzyme systems, such as mixed function oxidase (cytochrome p450 or p448 systems). Biotransformation of a xenobiotic to increase its water solubility for the purpose of enhancing excretion may generate reactive intermediates.
- interact chemically or physically with target cells or receptors (e.g., the chemical may interact with a biological receptor molecule or react with biological macromolecules and the interaction or reaction, such as oxidation or epoxidation, may result in disruption of normal biological function at that target site). In acute or repeated-dose toxicity studies this interaction may occur because the substance or a metabolite

⁹ A xenobiotic is any chemical compound that is foreign to a living organism.

mimics an endogenous substrate producing an adverse effect. In mutagenicity studies, this interaction may cause the impediment or alteration of DNA replication or a chemical reaction with DNA base pairs.

The similarity in physicochemical properties of the five alkyl sulfides which comprise this category suggest that the mammalian toxicity of all members of the category will be similar. Further, the structural features and properties of the members of the category suggest that the alkyl sulfides will be unable to reach target organs or, if they do, that they are not likely to produce toxic effects. The hydrophobic nature of these substances may favor penetration of biological membranes, but their subsequent transport via the circulatory system to target cell or receptors will be limited due to these same hydrophobic properties and the general absence of functional groups and reactive sites where biotransformation reactions might enhance hydrophilicity. The low volatility of these substances suggests that very low amounts of these materials will be available for absorption via inhalation. The high viscosity of these substances suggests that it will be difficult to generate high concentration of respirable particles in the air. Four of these compounds [i.e., methyl propene derivative (CAS # 68511-50-2), trimethyl pentane derivative (CAS # 68515-88-8), decene derivative (CAS # 67762-55-4), and C15-C18 alkene derivative (CAS # 72162-15-3)] consist of saturated carbon-carbon linkages and extremely stable polysulfide bonds; consequently, biological activation via epoxidation, O-insertion, or hydrolysis is thermodynamically unfavorable and is unlikely. In addition, these four substances lack functional groups that are potentially reactive or susceptible to hydrolysis as stated in Section 3.1.2.

The member of the category with the lowest molecular weight (propanol/dodecylthio derivative; CAS # 67124-09-8) contains a hydroxyl group.^{10,11} The hydroxyl group could be chemically reactive under some circumstances, but the level of delocalized electrons within the molecule stabilizes it. The saturated linkages limit the possibility of a strong sigma negative charge on the hydroxyl group (which is a weak electrophile). Furthermore, the hydroxyl group confers a greater degree of hydrophilicity. It is important to note that while the hydroxyl group provides a potential site of oxidation to form a carbonyl or carboxylic acid moiety, the hydroxyl group is also a target for glucuronidation and sulfation reactions. These types of biotransformation reactions are generally considered detoxification pathways that lead to inactivation of a potentially reactive site and enhance urinary excretion. Consequently, this substance is expected to share the same toxicological properties as the rest of the category. At the same time, these features (i.e., smallest member of the category, presence of the single, potentially reactive functional group within the category, and expected greatest degree of relative bioavailability within the category) are such that if any member of the category may show mammalian toxicity, it is more likely to be the propanol/dodecylthio derivative than any other in the category. Thus, it serves as a “marker chemical” that establishes the potential upper bound potential for mammalian toxicity for the entire group. Assuming

¹⁰ “The Use of Structure-Activity Relationships (SAR) in the High Production Volume Chemicals Challenge” Program (1999) EPA Chemical Right-to-Know website August 26, 1999.

¹¹ Federal Drug Administration, Toxicological Principles for the Safety Assessment of Direct Food Additives, Appendix 1.

the mammalian toxicity of this substance is similar to that of the remaining four, then the designation of these five alkyl sulfides as a category, as defined by EPA, is scientifically justified.¹²

3.3.2 Acute Mammalian Toxicity of the Alkyl Sulfide Category

3.3.2.1 Acute Toxicity Test Methodology

Acute toxicity studies investigate the effect(s) of a single exposure to a relatively high dose of a substance. Potential routes of exposure for acute toxicity assays include oral, dermal, and inhalation. Oral toxicity assays are conducted by administering test material to fasted animals (typically rats or mice) in a single gavage dose. Acute dermal toxicity tests are conducted by administering test material to the shaved skin on the back of the test animal (typically rats or rabbits) and allowing the test material to stay in contact with the skin application site for a specific duration (usually 24 hours). Acute inhalation toxicity assays are conducted by exposing test animals (typically rats) in a controlled atmosphere to a fixed air concentration of the test substance for a specific duration (typically 4 hours). The test material is either generated as a vapor or intentionally aerosolized into respirable particles, then metered into the exposure air at the desired concentration. Preferably, inhalation toxicity studies are conducted using either nose-only or head-only exposure to minimize potential confounding effects resulting from whole-body exposure. This methodology may lead to over-prediction of inhalation toxicity hazard (e.g., increasing the body-burden of the test material through skin absorption or ingestion (oral exposure) of test material as a consequence of grooming both during and after the intended exposure period).

Historically, lethality is a primary end-point of concern in acute toxicity studies and the traditional index of acute toxicity potency is the medial lethal dose that causes mortality in 50 percent of the test animals (LD₅₀). In acute inhalation studies, the traditional metric of potency is the median lethal concentration of the test material in air that causes mortality in 50 percent of the test animals (LC₅₀). In addition to lethality, acute toxicity studies also provide insights regarding potential systemic toxicity through careful observation and recording of clinical signs and symptoms of toxicity as well as through detailed examination of tissues and organ systems.

Typically, acute oral and dermal toxicity studies are conducted using a limit dose of 5000 and 2000 mg/kg body weight, respectively, and acute inhalation toxicity studies are conducted using a limit dose of 5 mg/L for 4 hours (according to OECD and EPA testing guidelines). Recently, harmonized EPA testing guidelines (August 1998) have set the limit dose for both oral and dermal acute toxicity studies at 2000 mg/kg body weight, while the recommended limit concentration for acute inhalation studies has been set at 2mg/L for 4 hours. The

¹²EPA guidance document: "Development of Chemical Categories in the HPV Challenge Program" (Last Revision, August 25, 1999).

limit dose test method minimizes the number of animals tested by exposing a single group of animals to a large dose (the limit dose) of the test substance. If less than 50 percent mortality is observed at the limit dose, no further testing is needed. A test substance that shows effects at a concentration greater than the limit dose is considered essentially nontoxic. If compound-related mortality is observed, then further testing may be necessary.

3.3.2.2 Summary of Available Data

Acute toxicity information considered adequate under the Program for the alkyl sulfide category are summarized in Table 5.

3.3.2.2.1 Acute Oral Toxicity

Acute oral toxicity studies in rats considered adequate under the Program are available on three of the five chemicals in the alkyl sulfide category: propanol/dodecylthio derivative (CAS # 67124-09-8), methyl propene derivative (CAS # 68511-50-2), and trimethyl pentane derivative (CAS # 68515-88-8). The acute oral toxicity tests for these chemicals were performed in accordance with OECD Guideline 401. The oral LD₅₀ for all three substances was greater than the 5,000 mg/kg limit dose indicating that these substances have relatively low toxicity.

Summary: All three compounds referenced above were essentially nontoxic upon acute oral exposure (i.e., LD₅₀ greater than the limit dose of 5000 mg/kg body weight), including the marker compound, propanol/dodecylthio derivative (CAS # 67124-09-8). In addition, the consistency of information on acute oral toxicity further supports treatment of all five alkyl sulfides as a chemical category within the HPV Program.

3.3.2.2.2 Acute Dermal Toxicity

Three of the substances in the alkyl sulfide category were adequately tested for use under the Program for acute dermal toxicity in rabbits: propanol/dodecylthio derivative (CAS # 67124-09-8), trimethyl pentane derivative (CAS # 68515-88-8), and C15-C18 alkene derivative (CAS # 67762-55-4). These studies were conducted in accordance with OECD Guideline 402. No mortality or treatment-related clinical signs of toxicity or gross lesions were observed for any substance when tested at the limit dose of 2000 mg/kg. Thus the dermal LD₅₀ was greater than 2000 mg/kg for all three substances, including the designated marker chemical within the group, the propanol/dodecylthio derivative (CAS # 67124-09-8).

Summary: Similar to the acute oral toxicity results, all three alkyl sulfides compounds referenced above were essentially nontoxic following acute dermal exposure (i.e., LD₅₀ greater than the limit dose of 2000 mg/kg body weight), including the marker compound, propanol/dodecylthio derivative (CAS # 67124-09-8). As with the acute oral toxicity results, the consistency of

data on acute dermal toxicity further substantiates treatment of all five alkyl sulfides as an HPV chemical category.

3.3.2.2.3 Acute Inhalation Toxicity

Two of the substances in the alkyl sulfide category have been adequately tested under the Program for acute inhalation toxicity: methyl propene derivative (CAS # 68511-50-2) and trimethyl pentane derivative (CAS # 68515-88-8). These tests were conducted according to OECD Guideline 403. The methyl propene derivative (CAS # 68511-50-2) was tested in rats, while the trimethyl pentane derivative (CAS # 68515-88-8) was tested in rats, mice, and guinea pigs.

In a study conducted with the methyl propene derivative (CAS # 68511-50-2), rats were exposed to a vapor of the test material at three concentrations. The highest dose tested was 0.39 mg/L with a whole-body exposure for 4 hours. No mortality was noted, and all animals fully recovered following depuration. No significant clinical signs were noted after the initial post-exposure observations at any dose level. No treatment-related macroscopic or microscopic findings were noted. The LC_{50} for the methyl propene derivative (CAS # 68511-50-2) was greater than 0.39 mg/L (highest dose tested).

Acute inhalation toxicity testing of the trimethyl pentane derivative (CAS # 68515-88-8), involved exposure to an aerosol of the test material. In one study, the LC_{50} was >5.6 mg/L for male rats and equal to 2.17 mg/L for female rats. No treatment-related gross lesions were noted in surviving rats.

Two other acute inhalation studies with aerosolized trimethyl pentane derivative (CAS # 68515-88-8) were conducted in rats, mice, and guinea pigs (rats, mice, and guinea pigs in one study and guinea pigs and mice in the second study). One study noted mortality in 3 out of 10 rats, 1 out of 10 mice, and 1 out of 10 guinea pigs at the maximum attainable concentration of 4.3 mg/L. No treatment-related clinical signs were noted for mice and guinea pigs. In another study in which mice and guinea pigs were also tested at the maximum attainable concentration of 4.3 mg/L, no mortality and no treatment-related clinical signs or gross lesions were noted. For mice and guinea pigs, the LC_{50} is considered to be >4.3 mg/L (maximum attainable concentration). The LC_{50} for male rats in this study is considered to be >4.3 mg/L, and the LC_{50} for females is <4.3 mg/L.

Summary: For the two compounds referenced above for acute inhalation toxicity, both exhibited relatively low toxicity (LC_{50} s were all above the maximum achievable concentration). The relatively low maximum achievable air concentrations are consistent with the low vapor pressures and the physical limitations of aerosolizing high viscosity materials. The administered dose via inhalation is very low compared to other routes of exposure (e.g., oral or dermal), thus decreasing the potential exposure of these

compounds via inhalation pathways. Inhalation is not considered a relevant route of exposure for the alkyl sulfides because of the limited volatilization and aerosolization potential of these compounds.

3.3.2.3 Data Assessment and Test Plan for Acute Mammalian Toxicity

In total, eleven acute toxicity studies considered adequate under the HPV Program have been conducted upon the alkyl sulfide chemical category. These studies have involved four different animal species; oral, dermal, and inhalation routes of exposure; and four of the five members of the category. The data consistently demonstrate low or no acute toxicity following acute oral, dermal or inhalation exposure. The HERTG conclude that the acute toxicity findings support the expectation, based on structural, physicochemical, and toxicological similarities, that these 5 compounds should be treated as a category. The HPV Challenge Program requires that either an acute oral, dermal, or inhalation test (preferably oral) be performed or bridged to each member chemical of a category. Although acute toxicity testing sufficient for the Program has not been conducted on the decene derivative (CAS # 72162-15-3), the structural features and physicochemical properties of the derivative are sufficiently similar to those of the other members of this category. Thus the decene derivative (CAS # 72162-15-3) is expected to exhibit acute toxicity findings similar to those of the other alkyl sulfides in this category, and, therefore, additional testing of the decene derivative (CAS # 72162-15-3) is not needed. The HERTG has concluded that the acute toxicity of the category has been evaluated adequately with respect to all acute toxicity HPV endpoints. No additional acute toxicity testing is proposed for purposes of the HPV Challenge Program.

3.3.3 Mutagenicity of the Alkyl Sulfide Category

3.3.3.1 Mutagenicity Test Methodology

Genetic toxicology is concerned with the effects of substances on genetic material (i.e., DNA and chromosomes). Within genetic material, the gene is the simplest functional unit whose essence is composed of DNA. Mutations are generally nonlethal, heritable changes to genes which may arise spontaneously or as a consequence of xenobiotic exposure. Genetic mutations are commonly measured in bacterial and mammalian cells. The simplest test systems measure the occurrence of a base-pair substitution mutation in which a single nucleotide is changed followed by a subsequent change in the complementary nucleotide on the other DNA strand. Frameshift mutations occur following the deletion or insertion of one or more nucleotides, which then changes the “reading frame” for the remainder of the gene or multiple genes. Genetic testing for these types of point mutations is generally accomplished by *in vitro* cellular assays for forward or reverse mutations. A forward mutation occurs when there is a detectable change in native DNA whereas a reverse mutation occurs when a mutated cell is returned to its initial phenotype. Both base-pair substitutions and frameshift mutations are routinely measured in bacterial cells by measuring the ability of a cell to acquire the capability to grow in an environment missing an essential amino acid. In

these tests, a large number of cells are examined to demonstrate a significant increase in the frequencies of mutations that occur over the frequency of spontaneous mutations.

Chromosomal aberrations are large scale numerical or structural alterations in eukaryotic chromosomes including deletions (visualized as breaks), translocations (exchanges), non-disjunction (aneuploidy), and mitotic recombination. Chromosomal breakage is the classical end point in chromosomal aberration assays. Substances that induce structural changes in chromosomes, especially chromosome breaks, are referred to as "clastogens." To visualize chromosomes and chromosomal aberrations following *in vitro* or *in vivo* treatment with a substance, cells are arrested in metaphase, treated to swell the chromosomes, fixed, transferred to slides and stained. The first metaphase following treatment is the time at which the greatest number of cells with damaged chromosomes may be observed. The most frequently used test systems investigate changes in mammalian cells (such as Chinese hamster ovary or lung cells; human or rat lymphocytes; or human, rat or mouse bone marrow cells) following either *in vitro* or *in vivo* exposure to the test substance. The micronucleus test is a common *in vivo* assay that measures the frequency of micronuclei formation (i.e., chromosomal fragments) in polychromatic erythrocytes.

3.3.3.2 Summary of Mutagenicity Data

A summary of the mutagenicity information for the alkyl sulfides is presented in Table 6.

3.3.3.2.1 Bacterial Gene Mutation Assay

The bacterial reverse mutation assay considered adequate under the HPV Program has been conducted on four of the substances in this category: propanol/dodecylthio derivative (CAS # 67124-09-8), methyl propene derivative (CAS # 68511-50-2), trimethyl pentane derivative (CAS # 68515-88-8), and C15-C18 alkene derivative (CAS # 67762-55-4). These tests were conducted according to OECD Guidelines 471 and/or 472.

For two of the test substances, methyl propene derivative (CAS # 68511-50-2) and trimethyl pentane derivative (CAS # 68515-88-8), various strains of *S. typhimurium* and *E. coli* were tested with and without metabolic activation at doses that included and/or exceeded the limit dose of 5000 µg/plate. For the other two test substances, propanol/dodecylthio derivative (CAS # 67124-09-8) and the C15-C18 alkene derivative (CAS # 67762-55-4), the highest dose tested using various strains of *S. typhimurium* was 1 µl/plate (highest dose at which no precipitation was formed) with and without metabolic activation. All tested chemicals were negative for mutagenic activity, with and without metabolic activation.

3.3.3.2.2 In vitro Chromosomal Aberration Assay

An *in vitro* chromosomal aberration assay (using Chinese hamster ovary cells) was conducted for the propanol/dodecylthio derivative (CAS # 67124-09-8). This study is considered adequate under the HPV Program and was conducted in accordance with OECD Guideline 473. The results of this study, performed with and without metabolic activation of the test material, were negative for clastogenicity.

3.3.3.2.3 *In vivo* Chromosomal Aberration Assays

In vivo chromosomal aberration assays (using bone marrow cells from mice that were dosed by oral gavage or intraperitoneal injection) were conducted with methyl propene derivative (CAS # 68511-50-2), trimethyl pentane derivative (CAS # 68515-88-8), and an analog of the products in this class (CAS # 91770-97-4). The analog is a C12-C16 alkyl sulfide (CAS # 91770-97-4) bearing a methyl substituted side chain, with a structure very similar to the other alkyl sulfides in this group. These studies are considered adequate under the HPV Program and were conducted in accordance with OECD Guideline 474. All test substances were negative for clastogenicity. One of the test substances, the methyl propene derivative (CAS # 68511-50-2) was also tested in an *in vivo* micronucleus assay in rats via the dermal route of exposure. The results of this study were also negative.

Summary: Either bacterial gene mutation assays, *in vitro* chromosomal aberration assays, or *in vivo* chromosomal aberration assays have been conducted for four members of the category. Neither mutagenicity nor clastogenicity was exhibited by any of the substances in the referenced tests.. These tests demonstrate a lack of DNA reactivity either in the absence or presence of metabolic activation. The lack of predicted reactivity of the propanol/dodecylthio derivative (CAS # 67124-09-8) due to the stability of the aliphatic carbon-thio linkage overrides any apparent potential detrimental effects conferred by the relatively low molecular weight and electron localization effect of the hydroxyl moiety. These findings support the expectation, based on the similar structural features and physicochemical properties, that all the compounds in the alkyl sulfide category do not react with DNA and chromosomes at the cellular level. In addition, the consistency of available data regarding genotoxicity further supports treatment of all five alkyl sulfides as a chemical category within the HPV Program.

3.3.3.3 Data Assessment and Test Plan for Mutagenicity

Members of the alkyl sulfide group have undergone one or more test for mutagenicity and/or clastogenicity considered adequate under the HPV Program. In total, eight adequate mutagenicity studies have been conducted upon various members of the alkyl sulfide chemical category. The assays have included bacterial mutation, *in vitro* chromosomal aberration, and *in vivo* chromosomal aberration studies in rats and mice, which have involved four of the five members of the alkyl sulfide category. Additionally, an analog of one of the members of this category has also been evaluated in an *in vivo* chromosomal aberration study

with mice. The data consistently demonstrate a lack of mutagenicity. These findings support the expectation, based on the similar structural features and physicochemical properties, that the compounds in the alkyl sulfide category are also similar in their lack of mutagenic potential.

The HPV Challenge Program requires that a gene mutation and a chromosomal aberration test be performed, or bridged, to each member chemical of a category. Although mutagenicity testing considered to be adequate for the HPV Program has not been conducted on the decene derivative (CAS # 72162-15-3), the similarity of the structural features and physicochemical properties of the derivative is similar to those of the other members of this category. Thus, the decene derivative (CAS # 72162-15-3) is expected to exhibit genotoxicity results similar to those of the other alkyl sulfides in this category and additional testing of the decene derivative (CAS # 72162-15-3) is not needed. No additional mutagenicity testing is proposed for purposes of the HPV Challenge Program.

3.3.4 Repeated Dose Toxicity of the Alkyl Sulfide Category

3.3.4.1 Repeated Dose Toxicity Test Methodology

Repeated dose toxicity studies evaluate the effect(s) of repeated exposure to a chemical over a significant period of the life span of an animal. Chronic repeated dose toxicity studies are concerned with potential adverse effects upon exposure over the greater part of an organism's lifespan (e.g., one to two years in rodents). Subchronic repeated dose studies are also concerned with effects caused by exposure for an extended period, but not one that constitutes a significant portion of the expected lifespan. Typically, the exposure regimen in a subchronic study involves daily exposure (at least 5 consecutive days per week) for a period of at least 28 days or up to 90 days (i.e., 4 to 13 weeks). The dose levels evaluated are notably lower than the relatively high limit doses used in acute toxicity studies. In general, these studies are designed to assess systemic toxicity but the study protocol can be modified to incorporate evaluation of potential adverse reproductive and/or developmental effects.

3.3.4.1.1 Systemic Organ Studies

These studies provide information on systemic effects of a test substance in laboratory animals (rats, rabbits, or mice) when exposed via oral, dermal, or inhalation routes of administration over a limited duration (usually 28-90 days). A two-week recovery period (generally included in most study designs) following completion of the dosing or exposure period provides information on whether or not the effects seen during the exposure period are reversible. These studies are useful in identifying target organ(s), and they can be used in selecting dose levels for longer-term studies and for further refining safety criteria for human exposure. An indicator of the toxicity of a substance when tested in subchronic studies is the NOAEL (no observed adverse effect level). This measurement, usually expressed in mg/kg/day, defines the dose of test material that produced no significant toxicological

effects. Should the test material produce toxicity at the lowest dose tested (i.e., no defined NOAEL), the lowest dose that produced an adverse effect is presented as the LOAEL.

3.3.4.1.2 Reproductive/Developmental Studies

Reproductive and developmental toxicity studies generate information on the effects of a test substance on male and female reproductive performance such as gonadal function, mating behavior, conception, and development of the conceptus, parturition, and post-partum development of the offspring. Various study designs exist, but they all involve exposure to both male and female test animals beginning before mating. The rat is most often selected as the test species. The test substance is administered to males and females continuously at several graduated doses for at least two weeks prior to mating and until the animals are sacrificed. The males are treated for at least two more weeks. Male gonadal histopathology is carefully assessed at the end of the study. The females are treated through parturition and early lactation. The adult females and offspring are typically studied until termination on post-natal day 21, or sometimes earlier. In addition to providing data on fertility and reproduction, this study design provides information on the potential for prenatal exposure, and limited post-natal exposure to affect development. NOAEL or LOAEL are also applicable to these tests, with the exception that these values are derived from effects specific to reproduction or development.

The “toxicity to reproduction” requirement in the HPV Challenge Program can be met by conducting a reproductive/developmental toxicity screening test or adding a reproductive/developmental toxicity screening test to the repeated dose study (OECD 421 or OECD 422, respectively). The one-generation reproduction toxicity study (OECD 415) is a more comprehensive protocol for the study of the effect of a test material on reproduction and development that also meets the SIDS and the HPV Program requirements.

3.3.4.2 Summary of Repeated Dose Toxicity Data

A summary of the results from the repeated dose studies considered adequate for the HPV Program for alkyl sulfides is presented in Table 7.

3.3.4.2.1 Systemic Toxicity Tests

The following subchronic systemic toxicity studies are applicable to the chemicals in the alkyl sulfide group under the HPV Program:

- A 28-day (oral) repeated dose study in rats (with 2-week reversibility period). Dose levels of 100, 300, or 1000 mg/kg/day.
(Test material: propanol/dodecylthio derivative (CAS # 67124-09-8)).
- A 13-week dermal administration to rats. Dose levels of 10, 50, 100, 250, 500, or 2000 mg/kg/day.

(Test material: methyl propene derivative (CAS # 68511-50-2))

- A 28-day subchronic dermal toxicity study in rabbits. Dose levels of 200 or 2000 mg/kg/day.
(Test material: methyl propene derivative (CAS # 68511-50-2))
- A 21-day repeated dermal application study in rabbits. Dose levels of 140, 560, or 2240 mg/kg/day.
(Test material: methyl propene derivative (CAS # 68511-50-2))
- A 28-day inhalation study in rats with a 3-week recovery period. Dose levels of 15, 50, or 150 mg/m³.
(Test material: trimethyl pentane derivative (CAS # 68515-88-8))
- A 28-day dermal toxicity study in rats. Dose level of 1,000 mg/kg/day.
(Test material: trimethyl pentane derivative (CAS # 68515-88-8))

As noted above and in Table 7, four subchronic dermal toxicity studies (in rats or rabbits) have been conducted with either the methyl propene derivative (CAS # 68511-50-2) or trimethyl pentane derivative (CAS # 68515-88-8). The predominant effect noted was dermal irritation at the site of test material administration. Among the four subchronic dermal toxicity studies conducted for this category, the lowest reported NOEL was 50 mg/kg/day for a 13-week rat dermal study with methyl propene derivative (CAS # 68511-50-2).

A 28-day oral toxicity study in rats has been conducted with the propanol/dodecylthio derivative (CAS # 67124-09-8) and a 28-day inhalation study in rats has been conducted with the trimethyl pentane derivative (CAS # 68515-88-8). In both studies, gross and microscopic observations at study termination showed alterations in kidneys and liver which were similar in nature for both test materials. Gross observations included increased kidney and liver weights in both studies. Microscopic alterations in the kidneys were seen primarily in male rats and consisted of increased incidences of globular casts and the presence of hyaline droplets in the proximal tubule cells. Although the globular casts were also seen after the recovery period, no hyaline droplets were seen in the recovery animals, indicating that this change was reversible after cessation of test substance administration. This effect is considered to be an organ-, gender- and species-specific (i.e., kidney, male, rat) response that is commonly observed following repeated administration of long-chain aliphatic hydrocarbon-based materials.^{13,14} The male rat is

¹³ Halder, C.A., et al., Renal Toxicity of Gasoline and Related Petroleum Napthas in Male Rats, *Renal Effects of Petroleum Hydrocarbons*, M.A. Mehlman, C.P. Hemstreet, J.J. Thorpe and N.K. Weaver, eds., Princeton Scientific Publishers, Inc., Princeton, N.J., pp 73-88, 1984.

considered uniquely sensitive to such effects, and these effects are considered irrelevant to humans (cite!). Microscopic examination of the liver showed hypertrophy of hepatocytes in all animals at termination of the dosing period and in the high-dose animals at the end of the recovery period. These alterations represent a compensatory increase in the activity of hepatic metabolic processes in response to a xenobiotic challenge, and normally are not considered to be pathological.

Summary: Repeated-dose toxicity tests have been conducted on three of the alkyl sulfide compounds. The results of these studies consistently demonstrate relatively low systemic toxicity among the three tested substances including the marker compound, propanol/dodecylthio derivative (CAS # 67124-09-8). The effects on the liver that have been noted in these studies are considered to be adaptive responses. The effects on the kidney in male rats observed in these studies are not relevant to humans.

3.3.4.2.2 Reproductive/Developmental Toxicity

No reproductive or developmental toxicity data considered adequate under the HPV Program for the alkyl sulfide category are available.

3.3.4.3 Data Assessment and Test Plan for Repeated Dose Toxicity

3.3.4.3.1 Systemic Toxicity

Six repeated dose systemic toxicity studies involving two different animal species and three of the five members of the alkyl sulfide category have been conducted. The results of these repeated-dose toxicity studies in various laboratory animal models collectively show no significant adverse biological responses or specific toxicity relevant to humans resulting from exposure to chemicals in the alkyl sulfide group. The observed effects to the kidney were considered to be specific to the male rat and without relevance to humans, and the adaptive changes to the liver are not considered to be adverse effects. The structural features and physicochemical properties of the decene derivative (CAS # 72162-15-3) and the C15-C18 alkene derivative (CAS # 67762-55-4) are sufficiently similar to those of the other members of this category. Thus, these derivatives are expected to exhibit repeated dose toxicity results similar to those of the other alkyl sulfides in this category and additional testing is not needed. The repeated-dose toxicity of the category has been evaluated adequately with respect to all repeated dose toxicity HPV endpoints. No additional repeated dose toxicity testing is proposed for purposes of the HPV Challenge Program.

¹⁴Goldsworthy, Thomas L., et al., Potential Role of α -2 I-Globulin, Protein Droplet Accumulation and Cell Replication in the Renal Carcinogenicity of Rats Exposed to Trichloroethylene, Perchloroethylene and Pentachloroethane, *Toxicology and Applied Pharmacology*, Volume 96, pp. 367-379, 1988.

3.3.4.3.2 Reproductive/Developmental Toxicity

Although the alkyl sulfide category is well tested for other health effects and the data demonstrate similarity of toxicity across all members of the category, no reproductive/developmental studies considered to be adequate under the HPV Program are available to the HERTG. Based upon the previously discussed similarities in physicochemical properties of the alkyl sulfide category, in combination with the existing testing data confirming that the category exhibits low biological activity, it is reasonable to expect that exposure to alkyl sulfides will not result in reproductive/developmental effects.

However, given the absence of any reproductive/developmental testing data considered adequate under the HPV Program for this group, HERTG will conduct a 1-generation reproductive toxicity test upon the propanol/dodecylthio derivative (CAS # 67124-09-8). As discussed at the beginning of this section, it serves as a “marker chemical” that establishes the potential upper bound potential of mammalian toxicity for the entire group. Reproductive/developmental testing of only this marker chemical is consistent with EPA guidance in the document titled “Development of Chemical Categories in the HPV Challenge Program.” This guidance, which states that “[I]n certain cases, such as where toxicity is low and no upward trend is expected, (emphasis added) extrapolation to the higher category members may be acceptable.” The studies for all endpoints other than reproductive/developmental toxicity demonstrate that toxicity is relatively low among the members of this category. Thus, the decision to evaluate reproductive toxicity by testing the propanol/dodecylthio derivative (CAS # 67124-09-8) is both scientifically justified and consistent with EPA guidance.

If testing of the propanol/dodecylthio derivative (CAS # 67124-09-8) shows low reproductive/developmental toxicity, no further testing of the category will be considered necessary.

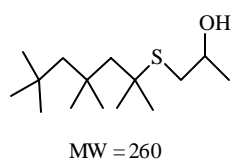
If the testing of the propanol/dodecylthio derivative (CAS # 67124-09-8) does yield test results that are positive or equivocal, the HERTG will evaluate the need for additional reproductive testing in this category. This stepwise approach to testing is scientifically justified, it is consistent with EPA guidance, and is preferred because it improves the quality of the subsequent research and reduces the unnecessary use of test animals.

Table 1. Members of the Alkyl Sulfide Category

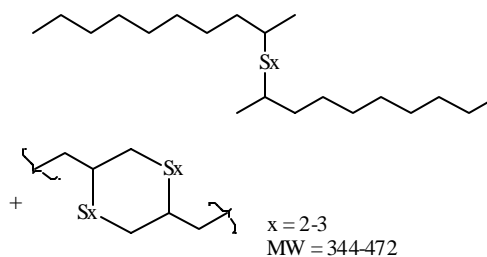
Chemical Name	Simplified Chemical Name	CAS Number
2-propanol, 1-(tert-dodecylthio)-	propanol/dodecylthio derivative	67124-09-8
1-decene, sulfurized	decene derivative	72162-15-3
1-propene, 2-methyl-, sulfurized	methyl propene derivative	68511-50-2
Pentene, 2,4,4-trimethyl-, sulfurized	trimethyl pentane derivative	68515-88-8
Alkenes, C15-18 alpha-, sulfurized	C15-C18 alkene derivative	67762-55-4

Figure 1. Chemical Structures

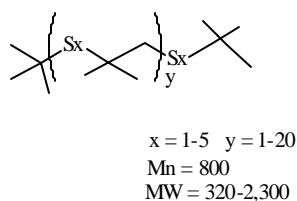
1. Propanol/dodecylthio derivative
CAS# 67124-09-8



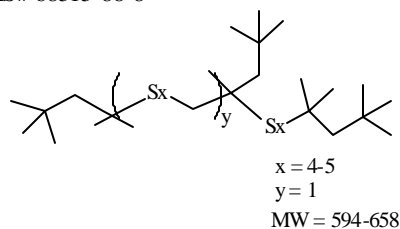
2. Decene derivative
CAS# 72162-15-3



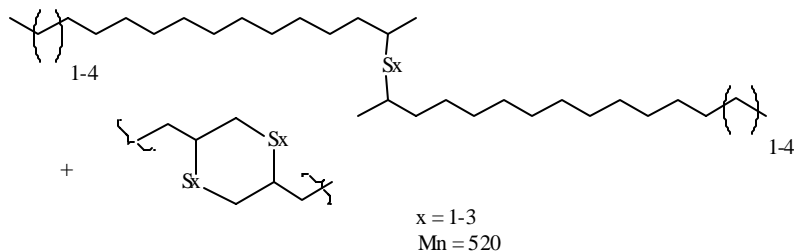
3. Methyl propene derivative
CAS# 68511-50-2



4. Trimethyl pentane derivative
CAS# 68515-88-8



5. C15-C18 alkene derivative
CAS# 67762-55-4



MW = molecular weight

Mn = number average molecular weight

Table 2. Functional Group, Chemical Classes, and Hydrolytic Potential of Alkyl Sulfide Category Compounds

Members of the Alkyl Sulfide Category	Functional Group and Chemical Class	Potential for Hydrolysis
2-propanol, 1-(tert-dodecylthio)-	Alcohol	Low
	Alkane	Low
	Sulfide	Low
1-decene, sulfurized	Alkene	Low
	Alkane	Low
	Sulfide	Low
	Polysulfide	Low
1-propene, 2-methyl-, sulfurized	Alkene	Low
	Alkane	Low
	Sulfide	Low
	Polysulfide	Low
Pentene, 2,4,4-trimethyl-sulfurized	Alkene	Low
	Alkane	Low
	Sulfide	Low
	Polysulfide	Low
Alkenes, C15-18, alpha, sulfurized	Alkene	Low
	Alkane	Low
	Sulfide	Low
	Polysulfide	Low

Table 3. Evaluation of Environmental Fate Information

CHEMICAL	BIODEGRADABILITY	HYDROLYSIS	PHOTODEGRADATION
	PROPOSED TESTING OR INFORMATION FOR ENDPOINT	PROPOSED TESTING OR INFORMATION FOR ENDPOINT	PROPOSED TESTING OR INFORMATION FOR ENDPOINT
propanol/dodecylthio derivative (CAS # 67124-09-8)	Adequate data (5.9% biodegraded after 28 days)	No testing needed technical limitations ¹	AOPWIN Model Estimation
decene derivative (CAS # 72162-15-3)	Test	No testing needed technical limitations ¹	No testing needed Bridging
methyl propene derivative (CAS # 68511-50-2)	Adequate data (0.3% biodegraded after 28 days)	No testing needed technical limitations ¹	AOPWIN Model Estimation
trimethyl pentane derivative (CAS # 68515-88-8)	No testing needed Bridging	No testing needed technical limitations ¹	No testing needed Bridging
C15-C18 alkene derivative (CAS # 67762-55-4)	No testing needed Bridging	No testing needed technical Limitations ¹	AOPWIN Model Estimation

¹See technical discussion included in hydrolysis section.

Table 4. Evaluation of Ecotoxicology Information

CHEMICAL	ACUTE TOXICITY TO FISH PROPOSED TESTING OR INFORMATION FOR ENDPOINT [96-HOUR LC50¹ (mg/L)]	ACUTE TOXICITY TO INVERTEBRATES PROPOSED TESTING OR INFORMATION FOR ENDPOINT [48-HOUR EC50¹ (mg/L)]	TOXICITY TO ALGAE PROPOSED TESTING OR INFORMATION FOR ENDPOINT [96-HOUR EC50¹ (mg/L)]
Propanol/dodecylthio derivative (CAS # 67124-09-8)	Test	Test	Test
Decene derivative (CAS # 72162-15-3)	No testing – bridging	No testing – bridging	No testing – bridging
Methyl propane derivative (CAS # 6811-50-2)	>1,000 (WAF ² , F) >10,000 (WSF ⁴ , S)	>1,000 (WAF ³ , D)	R >100 (WAF ³ , P) B = 34 (WAF ³ , P)
Trimethyl pentane derivative (CAS # 68515-88-8)	No testing – bridging	No testing – bridging	No testing – bridging
C15-C18 alkene derivative (CAS # 67762-55-4)	No testing – bridging	No testing – bridging	No testing – bridging

¹Toxicity endpoints for the chemicals are expressed as median lethal concentration (LC₅₀) for fish and median effective concentration (EC₅₀) for *Daphnia* and algae. The EC/LC₅₀ is defined as the concentration that adversely effects 50% of the test organisms exposed to the chemical during a specific time. The greater the EC/LC₅₀ the lower the toxicity. See report text for information regarding differences between reported LC₅₀ and EC₅₀ values and lethal loading rate (LL₀) and effective loading (EC₀) values.

²WAF = Water accommodated fraction static renewal test.

³WAF = Water soluble fraction static non-renewal test.

⁴WSF= Water soluble fraction static renewal test.

F = fathead minnow, *Pimephales promelas*.

D = freshwater cladoceran, *Daphnia magna*.

P = freshwater algae *Pseudokirchneriella subcapitata* formerly called *Selenastrum capricornutum*.

S = sheepshead minnow, *Cyprinodon variegatus*.

R = growth rate

B = biomass

Table 5. Evaluation of Acute Toxicity Information

CHEMICAL	ACUTE TOXICITY
	PROPOSED TESTING OR INFORMATION FOR ENDPOINT
propanol/dodecylthio derivative (CAS # 67124-09-8)	Data Available <ul style="list-style-type: none"> • Oral – LD50 >5000 mg/kg (rat) • Dermal – LD50>2000 mg/kg (rabbit)
decene derivative (CAS # 72162-15-3)	No testing needed <ul style="list-style-type: none"> • bridging
methyl propene derivative (CAS # 68511-50-2)	Data Available <ul style="list-style-type: none"> • Oral – LD50 >5000 mg/kg (rat) • Oral – LD50 = 5.7 ml/kg (rat) Inhalation – LC50 >0.39 mg/L (rat) (highest dose tested)
trimethyl pentane derivative (CAS # 68515-88-8)	Data Available <ul style="list-style-type: none"> • Oral – LD50>5000 mg/kg (rat) • Dermal – LD50 >2000 mg/kg (rabbit) • Inhalation – LC50 = >5.6 mg/L (male rat); 2.17 mg/L (female rat) • Inhalation – LC50 >4.3 mg/L (male rat, mouse, guinea pig); <4.3 mg/L (female rat) (maximum attainable concentration) Inhalation – LC50 >4.3 (mouse, guinea pig)
C15-C18 alkene derivative (CAS # 67762-55-4)	Data Available <ul style="list-style-type: none"> • Dermal – LD50>2000 mg/kg (rabbit)

Table 6. Evaluation of Mutagenicity Information

CHEMICAL	BACTERIAL GENE MUTATION ASSAY	IN VITRO CHROMOSOMAL ABERRATION ASSAY	IN VIVO CHROMOSOMAL ABERRATION ASSAY
	PROPOSED TESTING OR INFORMATION FOR ENDPOINT	PROPOSED TESTING OR INFORMATION FOR ENDPOINT	PROPOSED TESTING OR INFORMATION FOR ENDPOINT
propanol/dodecylthio derivative (CAS # 67124-09-8)	Adequate data Negative +/- S9	Adequate data Negative +/- S9	No testing needed • adequate data with <i>in vitro</i> test
decene derivative (CAS # 72162-15-3)	No testing needed • bridging	No testing needed • bridging	No testing needed • bridging
methyl propene derivative (CAS # 68511-50-2)	Adequate data Negative +/- S9	No testing needed • adequate data with <i>in vivo</i> test	Adequate data • Negative (mouse) Negative (rat)
trimethyl pentane derivative (CAS # 68515-88-8)	Adequate data Negative +/- S9	No testing needed • adequate data with <i>in vivo</i> test	Adequate data Negative (mouse)
C15-C18 alkene derivative (CAS # 67762-55-4)	Adequate data Negative +/- S9	No testing needed • adequate data with <i>in vivo</i> test	No testing needed • bridging
analog for C15-C18 alkene derivative (CAS # 91770-97-4)			Adequate data Negative (mouse)

Table 7. Evaluation of Repeated Dose Toxicity Information

CHEMICAL	REPEATED DOSE TOXICITY	REPRODUCTIVE/DEVELOPMENTAL TOXICITY
	PROPOSED TESTING OR INFORMATION FOR ENDPOINT	PROPOSED TESTING OR INFORMATION FOR ENDPOINT
propanol/dodecylthio derivative (CAS # 67124-09-8)	<p>Adequate Data</p> <ul style="list-style-type: none"> STUDY: 28-Day Oral (rat) Dose Levels: 100, 300, or 1000 mg/kg/day Effects: ≥ 100 mg/kg/day: increased liver and kidney weights; globular casts; hyaline droplets in proximal tubules; hypertrophy of hepatocytes NOEL: < 100 mg/kg/day (not defined in the report) <p>Test</p> <p>1-generation reproduction</p>	<p>Test</p> <p>1-generation reproduction</p>
decene derivative (CAS # 72162-15-3)	<p>No testing needed</p> <ul style="list-style-type: none"> bridging 	<p>No testing needed</p> <ul style="list-style-type: none"> bridging
methyl propene derivative (CAS # 68511-50-2)	<p>Adequate data</p> <ul style="list-style-type: none"> STUDY ONE: 13-Week Dermal (rat) Dose Levels: 10, 50, 100, 250, 500, or 2000 mg/kg/day Effects: ≥ 250 mg/kg/day: decreased body weight gain (males); decrease in RBC; increase in neutrophils; increase in spleen size and pigments in spleen; ≥ 100 mg/kg/day: increased production of WBC in spleen and bone marrow; $\geq 10\%$ (100 mg/kg/day) concentration: dermal irritation NOEL: 50 mg/kg/day (systemic) 10% (dermal irritation) <p>No testing needed</p> <ul style="list-style-type: none"> Adequate repeated dose data bridging repro data 	<p>No testing needed</p> <ul style="list-style-type: none"> Bridging

Table 7. Evaluation of Repeated Dose Toxicity Information (continued)

CHEMICAL	REPEATED DOSE TOXICITY	REPRODUCTIVE/DEVELOPMENTAL TOXICITY
	PROPOSED TESTING OR INFORMATION FOR ENDPOINT	PROPOSED TESTING OR INFORMATION FOR ENDPOINT
	<ul style="list-style-type: none"> STUDY TWO: 28-Day Dermal (rabbit) Dose Levels: 200 or 2000 mg/kg/day (intact and abraded skin) Effects: ≥ 200 mg/kg/day: severe skin irritation; 2000 mg/kg/day: decrease in body weight and food consumption (males) NOEL: < 200 mg/kg/day (not defined in the report) 	
methyl propene derivative (CAS # 68511-50-2)	<ul style="list-style-type: none"> STUDY THREE: 21-Day Dermal (rabbit) Dose Levels: 140, 560, or 2240 mg/kg/day Effects: ≥ 140 mg/kg/day: severe erythema and slight to moderate edema. Epithelial hyperplasia of the skin in all treated animals. NOEL: < 140 mg/kg/day (not defined in the report) <p>No testing needed</p> <ul style="list-style-type: none"> Adequate repeated dose data bridging repro data 	No testing needed Bridging

Table 7. Evaluation of Repeated Dose Toxicity Information (continued)

CHEMICAL	REPEATED DOSE TOXICITY	REPRODUCTIVE/DEVELOPMENTAL TOXICITY
	PROPOSED TESTING OR INFORMATION FOR ENDPOINT	PROPOSED TESTING OR INFORMATION FOR ENDPOINT
trimethyl pentane derivative (CAS # 68515-88-8)	<p>Adequate data</p> <ul style="list-style-type: none"> STUDY ONE: 28-Day Inhalation (rat) Dose Levels: 15, 50, or 150 mg/m³ Effects: ≥ 15 mg/ m³: Trend toward lower body weight gain (all males and two highest doses in females); increased kidney weights (all males only); globular casts in cortico-medullary junction; hyaline droplets in proximal tubules (all males and recovery high dose males); increased liver weight (high-dose males and females and mid-dose males); 150 mg/L: decrease in hemoglobin concentration NOEL: < 15 mg/ m³ (not defined in the report) <p>No testing needed</p> <ul style="list-style-type: none"> adequate repeated dose data bridging reproduction data 	<p>No testing needed</p> <ul style="list-style-type: none"> bridging

Table 7. Evaluation of Repeated Dose Toxicity Information (continued)

CHEMICAL	REPEATED DOSE TOXICITY	REPRODUCTIVE/DEVELOPMENTAL TOXICITY
	PROPOSED TESTING OR INFORMATION FOR ENDPOINT	PROPOSED TESTING OR INFORMATION FOR ENDPOINT
trimethyl pentane derivative (CAS # 68515-88-8)	<ul style="list-style-type: none"> STUDY TWO: 28-Day Dermal (rat) Dose Levels: 1000 mg/kg/day Effects: Irritating to rat skin at 1000 mg/kg/day; Dermal irritation included well-defined erythema, scabbed areas, and dry flaking skin. Minimal to mild multifocal eschar and mild multifocal hemorrhage in the underlying dermis. NOEL: < 1000 mg/kg/day <p>No testing needed</p> <ul style="list-style-type: none"> adequate repeated dose data bridging reproduction data 	<p>No testing needed</p> <ul style="list-style-type: none"> bridging
C15-C18 alkene derivative (CAS # 67762-55-4)	<p>No testing needed</p> <ul style="list-style-type: none"> bridging 	<p>No testing needed</p> <ul style="list-style-type: none"> bridging